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DNA repair efficiency in young hypertensive: Is lifestyle a prooxidant factor?

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ABSTRACT

Background: High prevalence of hypertension (HTN) is of major concern among the middle-aged population of Kerala. Understudied in the context of HTN is oxidative damage to DNA, which is caused by free radical assaults. The modern lifestyle associated with an unhealthy diet and lack of exercise plays a key role in oxidative stress induction. **Aims and Objectives:** The objective of this study was to assess the significance of oxidative stress and the effectiveness of DNA repair mechanisms in young hypertensives along with how it correlates with aspects of lifestyle. **Materials and Methods:** This prospective case–control study enrolled clinically proven hypertensive patients referred from the outpatient department of Hridayalaya, Institute of Preventive Cardiology, Thiruvananthapuram, to Genetika, a research center for Cytogenetic Studies in South Kerala. Hypertensive patients (n = 180) within the age group of 18–39 were compared with matched healthy controls (n = 140). Associated lifestyle factors were determined. **Results:** In comparison to controls, cases had noticeably higher mean levels of malondialdehyde (MDA) and mean break per cell ($P < 0.05$). There was a difference that was statistically significant ($P < 0.05$) between the patients and controls in terms of diet, exercise, physical activity, obesity, mental stress, alcohol, and tobacco use. On comparing MDA and mean break per cell values with lifestyle factors, a statistically significant difference was detected ($P < 0.05$). Observed a significant positive correlation of break per cell value with MDA ($P < 0.05$). **Conclusion:** The current study demonstrated increased DNA damage and decreased DNA repair efficiency in young hypertensive and was strongly associated with lifestyle factors. Appropriate measures such as regular BP checkups, regular exercises, and abstinence from alcohol and smoking are to be taken for the prevention and control of HTN.

Key words: Hypertension; Oxidative stress; Reactive oxygen species; Malondialdehye

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INTRODUCTION

In the world, hypertension (HTN) is now the primary modifiable risk factor for both cardiovascular disease (CVD) and all-cause death. Studies from different parts of India reported a high prevalence of HTN.[1] The overall prevalence is about 30% among adults in India, with urban and rural prevalence of 34% and 28%, respectively.[2] Although the prevalence of HTN was found to be more in an older population, currently an alarming increase is seen among the young adult population as observed in the HTN epidemiological study conducted in India. According to reports, the frequency of HTN is on the rise among young adults in Kerala, where the general population's prevalence is 30.4%.

HTN, being a silent disease, can quietly damage the body for years before symptoms develop.[3] Elevated blood pressure (BP) in young adults causes vascular damage and associated complications, ultimately leading to death. Recent studies reported that young adults with HTN are unaware that they have the condition, which may be the reason for hypertensive events in them. Hence, the focus must be on increasing awareness and control of BP levels.

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The number of young individuals with high BP is expected to escalate with lifestyle behaviors. Small-to-moderate-sized amounts of reactive oxygen intermediates or reactive oxygen species (ROI/ROS) generated during normal cellular metabolic activities might benefit physiological processes, including pathogen removal, wound healing, and tissue repair. However, disproportionate generation of ROI disrupts body homeostasis and results in pathological signaling as well as protein, lipid, and DNA damage. The primary producers of endogenous ROI are the several intracellular enzymes and the mitochondrial respiratory chain, whereas pollutants, cigarette smoking, alcohol consumption, and drugs are exogenous sources. Oxidative stress, characterized by excessive generation of ROS and disruption of redox signaling and control, induces vascular dysfunction and cardiovascular fibrosis in HTN.[4] DNA damage is a major factor in the advancement of coronary artery disease (CAD) and atherosclerosis. Oxidative stress, even at low levels induces extensive DNA damage that further leads to double-strand breaks and chromosomal aberrations.[5] Evidence from recent studies suggests that compared to those without HTN, HTN sufferers experience DNA damage more frequently.[6] Moreover, sustaining the integrity of the genetic blueprint depends on the effectiveness of DNA repair. Oxidative DNA damage and its repair have been of high interest for a long time.[7] Repair is feasible for mild DNA damage; nevertheless, necrotic, or programmed cell death may occur if the damage is severe and perhaps beyond the ability of repair mechanisms. Unrepaired DNA damage may have serious consequences leading to genomic instability.[8]

Many studies have examined the risk of HTN in adults, but to our knowledge, none of the studies has analyzed the effect of oxidative stress on DNA repair efficiency in young hypertensive and the role of lifestyle factors as prooxidants. Therefore, the goal of the current study is to assess how these factors contribute to HTN to prevent future complications associated with it.

Aims and objectives

The aim of the study is to investigate a possible link between lifestyle factors and DNA repair efficiency in young hypertensive. To evaluate DNA repair efficiency in young adults with hypertension and to correlate DNA repair efficiency with lifestyle factors.

MATERIALS AND METHODS

This prospective case–control study was conducted in clinically proven hypertensive patients referred from the outpatient department of Hridayalaya, Institute of Preventive Cardiology, Thiruvananthapuram, to Genetika, a cytogenetic diagnostic and research center in South Kerala. Institutional Ethics Committee (18/IEC/GTKA), November 22, 2015 authorized the study and compiled it with the Declaration of Helsinki and principles for effective clinical work. Of 180 clinically proved HTN young individuals (aged 18–39) were chosen as cases, whereas 140 individuals in good health who had been assigned for sex-matched and age have been selected as controls. Of the total 320 individuals, 172 were males and 148 were females. The study excluded patients with any kind of chronic sickness or other long-term conditions, as well as those who had previously been exposed to ionizing radiation, chemotherapy, or other mutagenic chemicals. Data were collected from the years 2015 to 2019. Lifestyle characters were recorded using pro forma. The purpose of the research was explained in detail to the participants before obtaining written consent.

Two mL of peripheral blood was collected aseptically by venipuncture from each participant. To measure the DNA repair efficiency using an *in vitro* mutagen sensitivity study, 1 mL of blood was transmitted using a sodium heparinized vacutainer. Following this, the remaining 1 mL of blood was placed into a plain tube and left to coagulate. Following a quick separation, the serum was used to analyze malondialdehyde (MDA), an oxidative stress marker by thiobarbituric acid (TBA) method according to a modified version of Yagi's and Satoh's (1984).

DNA repair–mutagen-induced sensitivity analysis (Hsu et al., 1987)

Bleomycin-induced mutagen sensitivity analysis was performed on each sample to assess the DNA repair efficiency of the subjects. In a 15 mL culture tube, add six drops of blood that have been heparinized and 10 mL of RPMI 1640 extension media that has been enhanced with 20% fetal calf serum. Phytohemagglutinin was added to the medium at a concentration of 10 μg/mL. After being kept at 37°C for 72 h, the cultures were kept in an incubator. Bleomycin (0.03 units/mL) was given to the cells during the 66th h. A single droplet of colchicine (0.04 μg/mL) was added at the 70th h. Once the contents had been incubated for 2 h, they were all transferred into a sterile centrifuge tube and centrifuged for 10 min at 1000 RPM. After being treated for 3 min with a hypotonic solution (0.075M KCl), a new fixative solution (Methanol: Acetic acid, 3:1) was used to fix the cells. The cells were placed on the little sliders, after which they were allowed to oxygen dry and stained with a 10% Giemsa solution. There was a visible chromatid breakage during chromosomal metaphases. DNA repair capacity may be evaluated using cytogenetic tests using mutagens. The repair mechanism becomes increasingly

deficient as the number of individual breaks left unrepaired increases. To determine the average break/cell value, the total amount of chromatid breaks has been divided by the total amount of metaphases. A break per cell value of more than one is considered hypersensitive.

MDA by TBA method

One mL serum was added to a 2 mL mixture of TBA – trichloroacetic acid – hydrochloric acid. Mixed thoroughly and boiled for 15 min in a water bath. After cooling in cold water, centrifuged at 3000 rpm for 10 min. The supernatant was taken and recorded the absorbance at 540 nm using a spectrophotometer.

Statistical analysis

The software Statistical Package for Social Sciences was used to do the statistical analysis (details). To compare the quantitative variables between the two groups, the independent sample "t" test was employed. The odds ratio and 95% confidence interval (CI) were used to quantify the strength of association, and the Chi-square test was employed for comparing the variables that are qualitative in nature.

A comparison of mean break per cell value and MDA with lifestyle factors was analyzed. The outcomes were displayed using the mean and standard deviation. For the purpose of statistical significance, a P<0.05 was used.

RESULTS

The 180 young people with HTN, ages 18–39, who participated in the current study were matched for age and sex with 140 healthy controls.

The mean levels of break per cell value and MDA in cases and controls are given in Table 1. Cases exhibited significantly higher mean values of MDA and mean break per cell values compared to controls (P<0.05).

A comparison of lifestyle factors between cases and controls is shown in Table 2. Diet, regular exercise, physical activity, obesity, mental stress, smoking, and alcohol consumption showed a P<0.05 indicating a difference that is statistically significant. To evaluate the strength of the correlation between the HTN and respective factors, the odds ratio of each variable was computed.

The association between lifestyle characteristics and mean break per cell value by univariate analysis is given in Table 3. On comparing the mean break per cell value with lifestyle factors, a statistically significant association was observed for diet, regular exercise, physical activity, obesity, mental stress, smoking, and alcoholism (P<0.05).

Table 4 shows the univariate analysis of MDA with lifestyle factors. On comparing MDA with lifestyle factors, an observable statistical difference is noticed for diet, regular

Table 1: Comparison of mean break per cell value and MDA in cases and controls

Parameters	Cases (n=180)		Controls (n=140)		T	P-value
	Mean	SD	Mean	SD		
Mean b/c value	0.813	0.081	0.664	0.041	19.773	<0.001
MDA	3.07	0.38	1.26	0.52	35.828	<0.001

Data as mean±standard deviation, MDA: Malondialdehyde, t indicates, P is the probability value

Table 2: Lifestyle factors between cases and controls

Category	Variables	Case–control				Total		Chi-square	P-value	OR	95% CI for OR	
		n	%	n	%	n	%				Lower	Upper
Diet	Vegetarian	41	22.8	9	6.4	50	15.6	15.97	<0.001	0.233	0.109	0.498
	Non-vegetarian	139	77.2	131	93.6	270	84.4					
Regular exercise	Yes	72	40	93	66.4	165	51.6	22.02	<0.001	0.34	0.21	0.53
	No	108	60	47	33.6	155	48.4					
Physical activity	Poor	59	32.8	23	16.4	82	25.6	11.045	<0.001	2.48	1.43	4.27
	Good	121	67.2	117	83.6	238	74.4					
Obesity	Yes	64	35.6	9	6.4	73	22.8	37.942	<0.001	8.03	3.83	16.85
	No	116	64.4	131	93.6	247	77.2					
Mental Stress	Yes	79	43.9	7	5	86	26.9	60.602	<0.001	14.86	6.58	33.58
	No	101	56.1	133	95	234	73.1					
Smoking	Yes	48	26.7	10	7.1	58	18.1	20.228	<0.001	4.73	2.29	9.74
	No	132	73.3	130	92.9	262	81.9					
Alcohol consumption	Yes	69	38.3	7	5	76	23.8	48.318	<0.001	11.81	5.22	26.74
	No	111	61.7	133	95	244	76.3					

Values expressed as number (percent), Chi-square test for categorical variables, CI: Confidence interval, OR: Odds ratio

Table 3: Univariate analysis of mean break per cell value with lifestyle factors

Category	Variables	n	Mean b/c value		P-value
			Mean	SD	
Obese	Yes	71	0.773	0.099	0.014
	No	249	0.741	0.098	
Diet	Vegetarian	50	0.785	0.093	0.004
	Non-vegetarian	270	0.741	0.099	
Regular exercise	Yes	165	0.730	0.096	0.001
	No	155	0.767	0.099	
Physical activity	Poor	82	0.784	0.098	<0.001
	Good	238	0.735	0.097	
Mental stress	Yes	86	0.801	0.090	<0.001

SD: Standard deviation

Table 4: Univariate analysis of MDA with lifestyle factors

Category	Variables	n	MDA		P-value
			Mean	SD	
Obese	Yes	71	2.74	0.81	<0.001
	No	249	2.14	1.01	
Diet	Vegetarian	50	2.70	0.78	0.001
	Non-vegetarian	270	2.20	1.02	
Regular exercise	Yes	165	2.03	1.00	<0.001
	No	155	2.54	0.94	
Physical activity	Poor	82	2.66	0.93	<0.001
	Good	238	2.14	0.99	
Mental stress	Yes	86	2.89	0.68	<0.001
	No	234	2.05	1.01	
Smoking	Yes	58	2.79	0.85	<0.001
	No	262	2.16	1.00	
Alcohol consumption	Yes	76	2.86	0.71	<0.001
	No	244	2.09	1.01	

MDA: Malondialdehyde, SD: Standard deviation

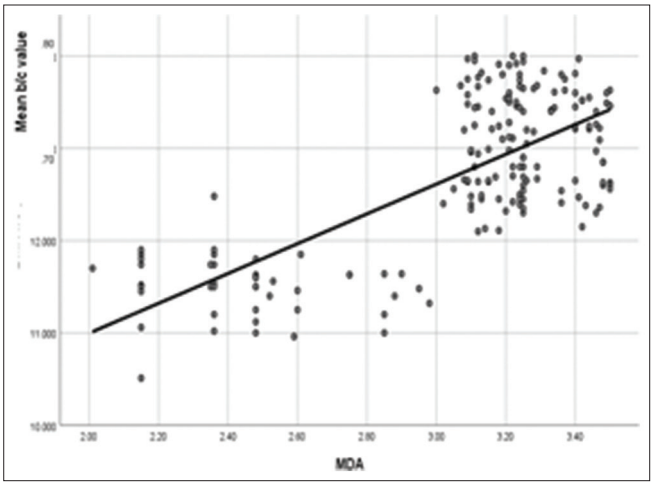

Figure 1: Pearson correlation of mean break per cell value with malondialdehyde

exercise, physical activity, obesity, mental stress, smoking, and alcoholism (P<0.05).

Figure 1 shows the Pearson correlation of mean break per cell value with MDA. A significant positive correlation of break per cell value with MDA is observed (P<0.05).

DISCUSSION

In the present study, hypertensive subjects exhibited higher levels of MDA than non-hypertensive, demonstrating increased oxidative stress in young hypertensive. The mean break per cell value was notably greater in cases (0.813) compared to controls (0.664), indicating increased DNA damage and decreased DNA repair efficiency in young hypertensives. A strong correlation between HTN and an increase in the mean break per cell value was discovered (P<0.05). Numerous research on animals support the idea that elevated BP is linked to increased oxidative stress; however, results from human studies have been inconsistent.[9]

Experimental and human studies by Paravicini and Touyz, 2006 demonstrated oxidative stress as a result of ROS overproduction, reduced nitric oxide, and antioxidant bioavailability as fundamental mechanisms responsible for the development of raised BP.[10] Moreover, Russo et al., 1998, observed increased lipid peroxidation in essential HTN.[11] A similar pattern of results was obtained in a study by Simon et al., 2013 reporting increased oxidative

stress and decreased effectiveness of DNA repair in CAD patients.[12] However, in line with the ideas obtained from previous findings and based on our results, it can be concluded that DNA damage and strand breaks due to oxidative stress are frequently occurring events in young hypertensive.

This is the first study from South India, to the best of our knowledge, assessing DNA repair efficiency and its association with lifestyle factors in young adults with HTN.

Based on population studies, the risk for cardiovascular complications increases about 2–3 times in individuals with HTN. The prognosis of the patient depends on the total of all the risk factors rather than the raised BP. Hence, the current study examined the association between each lifestyle risk factor with DNA damage and repair efficiency. In this investigation, we found that one of the main risk factors for HTN in young was obesity. Among the young adults, 73 (22.8%) were obese: 64 (35.6%) were hypertensive and 9 (6.4%) were non-hypertensive. The odds ratio of young adults being hypertensive is 8.03 suggesting obese are associated with higher risk of developing HTN than non-obese. One major risk factor for the development and exacerbation of HTN and heart disease is obesity, as per the study by Gajalakshmi et al.[13] Our findings are directly in line with previous findings that reported high oil and fat intake, fast foods, sedentary habits, and poor sleep patterns have been implicated in the development of obesity in young individuals.

Lifestyle elements, including nutrition and infrequent exercise, mental stress, habit of smoking, and alcohol consumption, had a strong association with HTN in this study. More than half of the subjects depended on a non-vegetarian type of diet (odds ratio [OR]=0.233). Results were similar to that obtained in a study by Matsumoto et al., 2019 that observed vegetarians have lower CVD risk factor levels and less prevalent CVD than non-vegetarians.[14]

Physical inactivity is an important modifiable risk factor in the advancement of HTN. The present study observed that those subjects involved in regular exercise were less likely to have HTN (OR=0.34) as regular exercise reduces sympathetic activity. This is consistent with what has been found in previous data from the CARDIA and ACLS studies, which reported an inverse association between cardiorespiratory fitness and HTN. Subjects who have irregular exercise had more mean break per cell value compared to those having regular exercise, indicating more DNA damage and decreased effectiveness of DNA repair due to increased oxidative stress. Regular exercise seems to stimulate the antioxidant system and the oxidative DNA damage-repairing system is upregulated. [15]

The knowledge regarding the mechanisms behind the protective effect of physical activity on hypertensive heart remains limited. The reduction in BP with physical activity and exercise may be due to a reduction in oxidative stress, inflammation, endothelial function, parasympathetic activity, renal function, and insulin sensitivity.[16] Regular exercisers are more likely to have better plasma lipoprotein profiles, decreased BP, and greater insulin sensitivity as per the study by Nystoriak and Bhatnagar).[17]

The body, when under stress, starts to release hormones and these hormones cause the heart to beat faster and the blood vessels to be narrower. Although these actions are said to increase BP, no proof that stress alone causes increased BP has been established. Stress can manifest as unhealthy lifestyle habits that can ultimately impact one's cardiovascular risk. Poor sleep, little or no exercise, unhealthy diet, smoking, drinking, and drug abuse can lead to higher BP and increase one's risk of stroke, or other heart issues. Based on the findings, the current study demonstrated an association (OR=14.86, 95% CI=6.58–33.58) between stress and HTN. A similar study by Liu et al. reported a positive association between mental stress and HTN.[18]

Cigarette smoking promotes endothelial dysfunction, which is a contributing factor for atherosclerosis. Jatoi et al. found that patients with essential HTN who smoke experience negative effects on their arterial stiffness parameters.[19] The present study also found a strong association between smoking and HTN (OR=4.73). A study by Solak et al. analyzed that cigarette smoking is associated with increased lipid peroxidation.[20] The mean break per cell value was elevated in smokers than non-smokers in our study, demonstrating decreased DNA repair efficiency due to increased DNA damages and increased oxidative stress in young hypertensives.

Excessive alcohol consumption and HTN have been linked in a number of epidemiological, pre-clinical, and clinical studies. In the present study, nearly 38.3% of the subjects among the test group were alcoholic and there was a positive correlation between alcoholic consumption and HTN (OR=11.81; 95% CI=5.22–26.74), which is supported by Suresh et al., who identified that alcohol abuse is a frequent contributor to elevated BP.[21] Mean break per cell values also showed a statistically significant difference (P<0.001) indicating decreased DNA repair efficiency in alcoholics.

Limitations of the study

A large study including more number of subjects with various lifestyle factors is required for clarifying the results of the study. Also, a study on genetic polymorphism of

DNA repair genes XRCC 1 and XRCC 3 may be useful in preventing complications of hypertension.

CONCLUSION

The current study demonstrated increased DNA damage and decreased DNA repair efficiency in young hypertensive and was strongly associated with lifestyle factors. Appropriate measures such as regular BP checkups, regular exercises, and abstinence from alcohol and smoking are to be taken for reducing oxidative stress and prevention and control of HTN.

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I confirm that all authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission.

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Author's Contribution:
SC- Designed the study, carried out the work, and analyzed the data; **SPPS**- Drafted the manuscript with input from all 2 authors and contributed to the interpretation of the result; **RG**- Provided critical feedback and helped to shape the research.

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